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Glutamate Is the Major Anaplerotic Substrate in the Tricarboxylic Acid Cycle of Isolated Rumen Epithelial and Duodenal Mucosal Cells from Beef Cattle[1,2]

Samer W. El-Kadi,[3] Ransom L. Baldwin VI,[4] Kyle R. McLeod,[5] Nishanth E. Sunny,[3] and Brian J. Bequette[3]*

[3]Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742; [4]Bovine Genomic Laboratory, Animal and Natural Resources Institute, USDA-Agricultural Research Service, Beltsville, MD 20705; and [5]University of Kentucky, Lexington, KY 40546

Abstract

In this study, we aimed to determine the contribution of substrates to tricarboxylic acid (TCA) cycle fluxes in rumen epithelial cells (REC) and duodenal mucosal cells (DMC) isolated from Angus bulls ($n = 6$) fed either a 75% forage (HF) or 75% concentrate (HC) diet. In separate incubations, [$^{13}C_6$]glucose, [$^{13}C_5$]glutamate, [$^{13}C_5$]glutamine, [$^{13}C_6$]leucine, or [$^{13}C_5$]valine were added in increasing concentrations to basal media containing SCFA and a complete mixture of amino acids. Lactate, pyruvate, and TCA cycle intermediates were analyzed by GC-MS followed by ^{13}C-mass isotopomer distribution analysis. Glucose metabolism accounted for 10–19% of lactate flux in REC from HF-fed bulls compared with 27–39% in REC from HC and in DMC from bulls fed both diets ($P < 0.05$). For both cell types, as concentration increased, an increasing proportion (3–63%) of α-ketoglutarate flux derived from glutamate, whereas glutamine contributed <3% ($P < 0.05$). Although leucine and valine were catabolized to their respective keto-acids, these were not further metabolized to TCA cycle intermediates. Glucose, glutamine, leucine, and valine catabolism by ruminant gastrointestinal tract cells has been previously demonstrated, but in this study, their catabolism via the TCA cycle was limited. Further, although glutamate's contribution to TCA cycle fluxes was considerable, it was apparent that other substrates available in the media also contributed to the maintenance of TCA fluxes. Lastly, the results suggest that diet composition alters glucose, glutamate, and leucine catabolism by the TCA cycle of REC and DMC. J. Nutr. 139: 869–875, 2009.

Introduction

The gastrointestinal tract (GIT)[6] of ruminants represents <10% of body weight (1), yet the metabolic activity of the GIT accounts for 25–35% of whole-body protein synthesis (2) and 20–40% of whole-body energy metabolism (3,4). Clearly, metabolism by the GIT has an important impact on whole-body energy requirements and, in this connection, on the availability of dietary nutrients to postabsorptive tissues for productive purposes (e.g. tissue gain, milk production).

It has been universally observed that the branched-chain amino acids (BCAA), glutamate, glutamine, and glucose are net removed in large quantities by the gut tissues of monogastric (5–8) and ruminant species (9–15). A question that is central to our understanding of the basis of energy requirements is: what pathways do substrates follow during their metabolism by the GIT tissues? Based on studies in rats (16–18) and piglets (5,6), a considerable portion of the net removal of amino acids (AA) and glucose by the gut tissues involves complete and (or) partial catabolism. In the piglet studies, the majority of CO_2 production by the intestinal mucosa derived from oxidation of AA (50–70%), with glucose contributing slightly less (30–40%). There is also synthesis and release by the portal-drained viscera (PDV; stomach, intestines, pancreas, and spleen) of 3-carbon end products, namely lactate and alanine, whose release by the PDV of piglets amounts to one-third of the sum of metabolized glutamate, glutamine, and glucose, with the remainder metabolized to CO_2 (6). In ruminants, such information is not complete and, given the larger range of substrates normally available to ruminant gut tissues, there may be greater metabolic plasticity. Nonetheless, knowledge of the sources of CO_2 and 3-carbon products generated by the gut tissues, and the dietary substrates metabolized to maintain anaplerotic and cataplerotic fluxes of the tricarboxylic acid (TCA) cycle,

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[6] Abbreviations used: AA, amino acid; BCAA, branched-chain amino acid; DMC, duodenal mucosal cell; FC, fractional contribution; GIT, gastrointestinal tract; HC, high concentrate; HF, high forage; KIC, ketoisocaproic acid; KIV, ketoisovaleric acid; KRB, Krebs ringer salt; MIDA, mass isotopomer distribution analysis; PDV, portal-drained viscera; REC, rumen epithelial cell; tBDMS, tertiary-butyldimethylsilyl; TCA, tricarboxylic acid; TTR, tracer:tracee ratio.
* To whom correspondence should be addressed. E-mail: bbequett@umd.edu.

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are central to our understanding of substrate flows through the gut tissues and the metabolic flexibility of these tissues when substrate composition is varied.

The first objective of this study was to determine the routes of metabolism and the extent that the catabolism of glucose, glutamate, glutamine, leucine, and valine contribute to TCA cycle fluxes in isolated rumen epithelial cells (REC) and duodenal mucosal cells (DMC). With the use of [U-^{13}C] tracers and application of ^{13}C-mass isotopomer distribution analysis (MIDA), we were able to quantify the contribution of these substrates to the fluxes through key entry points of the TCA cycle and, by difference, the contribution of other media substrates to TCA cycle fluxes. A second objective was to compare substrate metabolism by REC and DMC isolated from beef bulls fed either a high-forage (HF) or a high-concentrate (HC) diet, feeding situations where previous studies in vivo have observed major differences in net metabolism of AA and glucose by the PDV tissues (6,19,20). To further investigate diet influences on the regulation of substrate metabolism, we also quantified gene expression of regulatory enzymes by the REC and DMC isolated from the bulls in the current study (21).

Materials and Methods

Bulls and diets. The experimental protocol was approved by the Beltsville Area Animal Care and Use Committee (USDA) and by the Institutional Animal Care and Use Committee at the University of Maryland. Twelve Angus bulls were housed in individual stalls, adapted to their environment, and fed orchard grass silage for 1 wk prior to the initiation of the experiment.

Bulls were assigned to 2 groups ($n = 6$) based on body weight (mean in each group = 391 ± 34 kg). Each group was assigned to either a HF (75% orchard grass silage and 25% concentrate) or a HC (25% orchard grass silage and 75% concentrate) diet (**Table 1**) for at least 4 wk before slaughter. Diets were fed in amounts to meet requirements (22) for dry matter and energy intake to support 0.5 kg body weight gain/d. Fresh water was freely available by automatic waterers and feed refusals were weighed daily before the morning feeding. Bulls were weighed once per week throughout the experiment and feed intake was adjusted accordingly. By design, daily dry matter intake was similar in the 2 rations, resulting in 33% greater metabolizable energy intake by bulls fed the HC ration and mean daily body weight gains that were similar for bulls fed both rations (1.58 kg/d) (21). Thus, differences in substrate metabolism by the isolated gut cells reflect effects on the gut tissues of substrate and/or metabolizable energy supply, but not dry matter intake.

Isolation of REC and DMC. Bulls were stunned by captive-bolt, immediately exsanguinated, and the entire GIT removed from the abdominal cavity within 15 min. A 15- × 30-cm section of rumen wall was dissected from the cranial sac. The section was rinsed with warm tap water to remove feed particles and debris followed by rinsing in warm isotonic buffer [Krebs ringer salts (KRB)-HEPES]. The epithelial layers were stripped from the musculature, cut into 2- × 5-cm strips, and transported to the laboratory in insulated containers containing trypsin/$CaCl_2$ (5%; 0.016%) in warm KRB-HEPES.

REC were isolated from the remaining tissues following the procedures developed in our laboratory (23). Cell yield and viability were assessed using the trypan blue dye exclusion method (23).

Duodenal mucosal cells were collected from a segment 1 to 2 m distal to the pyloric sphincter. The segment was emptied of digesta, rinsed with warm tap water followed by KRB-HEPES buffer, and then cut longitudinally. Mucosa were scraped from the underlining musculature with a glass microscope slide and minced into 2- × 5-mm pieces. Scraped mucosa were transported to the laboratory in insulated containers containing collagenase/dispase I/$CaCl_2$ (90,000 collagenase digestive unit/L; 600 U/L; 140 mg/L) dissolved in freshly oxygenated warm KRB-HEPES. Mucosa cell isolation and tests of cell yield and viability were performed similar to procedures described for rumen epithelia (23).

TABLE 1 Ingredients and nutrient composition of the experimental diets

Ingredient	HF	HC
	g/kg dry matter	
Orchard grass, silage	750	250
Ground corn	167	554
Soybean meal	59	172
Calcium carbonate	12	14
Dicalcium phosphate	2	—
Vitamin mix[1]	5	5
Mineral mix[2]	5	5
Nutrient composition (calculated)		
Crude protein	137	174
Starch	130	419
Acid detergent fiber	333	140
Neutral detergent fiber	554	245
Crude fat	39	38
Metabolizable energy, *MJ/kg*	8.8	11.8

[1] Mineral mix provided g/kg: calcium, 220; salt, 160; sulfur, 31; phosphorus, 30; magnesium, 27; potassium, 24; iron, 1.82; zinc, 2.7; manganese, 024; iodine, 0.04; cobalt, 0.035; and selenium, 0.024.

[2] Vitamin mix provided g/kg: retinyl acetate, 1.82; cholecalciferol, 0.033; d,l-α-tocopherol acetate, 5.

Cell incubation. Isolated cells were incubated in 25-mL Erlenmeyer flasks containing a basal mixture of AA and SCFA (acetate, propionate, and butyrate) (**Table 2**) in KRB-HEPES. In separate incubations, [$^{13}C_6$]glucose, [$^{13}C_6$]leucine, [$^{13}C_5$]valine, [$^{13}C_5$]glutamate, or [$^{13}C_5$]glutamine was included in the basal mixture. [$^{13}C_6$]Glucose was added to unlabeled glucose at 20 mol/100 mol of unlabeled glucose. For all other incubations, [$^{13}C_6$]leucine, [$^{13}C_5$]valine, [$^{13}C_5$]glutamate, or [$^{13}C_5$]glutamine replaced 100% of the unlabeled AA (i.e. only tracer added). Incubations were performed at final concentrations of 0.25, 0.5, 1, or 2.5 times the basal blood concentrations of glucose, leucine, valine, glutamine, and glutamate (Table 2) except for REC incubation media, which contained SCFA in concentrations typically found in rumen fluid of beef cattle (14,24–26). All incubations were performed in triplicate.

Incubation flasks were purged for 20 s with O_2:CO_2 (95:5) and incubations initiated by the addition of 0.5 mL of the stock cell suspension. Immediately, flasks were sealed with a rubber serum cap and incubated in a reciprocal water bath (Precision Model 50, Jouan) at 37°C for 90 min. Incubations were terminated by the addition of 0.2 mL perchloric acid (70%; wt:wt) followed by neutralization with 0.4 mL potassium carbonate (5.8 mol/ L). Flask contents were transferred to borosilicate tubes and centrifuged at 2500 × *g*; 10 min and the supernatant decanted to clean tubes and stored at −20°C for later analysis.

MS of metabolites. ^{13}C-Enrichment of TCA cycle intermediates were analyzed by GC-MS (27) followed by ^{13}C-MIDA as previously described (28). To thawed samples was added sulfosalicylic acid (10% final volume) and the samples were centrifuged at 3000 × *g*; 5 min. The supernatant was decanted to clean screw-cap tubes, 5 mmol of hydroxylamine-hydrochloride added, and the pH adjusted to 7–8 with 4 mol/L potassium hydroxide. Samples were sonicated for 15 min with heat and allowed to react at 65°C for 1 h. Next, the pH was reduced to <2 by adding hydrochloric acid (6 mol/L) and the solution was saturated with NaCl prior to vortex mixing for 1 min to precipitate sulfosalicylic acid. The supernatant was extracted twice with 4 mL of ethyl acetate for 15 min and the extracts combined. The ethyl acetate extracts were centrifuged at 2000 × *g*; 5 min and the organic phase collected into V-vials. Samples were dried under a stream of N_2 gas at 40°C prior to forming the tertiary-butyldimethylsilyl (tBDMS) derivative.

TABLE 2 Nutrient composition of basal media used in primary cell incubations[1]

	DMC[2]	REC[2]
Experimental substrate concentrations	*mmol/L*	
Glucose	4	4
L-Leucine	0.23	0.23
L-Valine	0.33	0.33
L-Glutamine	0.15	0.15
L-Glutamate	0.27	0.27
Basal SCFA mixture		
Acetate	0.88	64
Propionate	0.04	23
Butyrate	0.01	15
Basal AA mixture		
L-Histidine	0.085	0.085
L-Isoleucine	0.144	0.144
L-Lysine	0.141	0.141
L-Methionine	0.032	0.032
L-Phenlalanine	0.074	0.074
L-Threonine	0.124	0.124
L-Tryptophan	0.022	0.022
L-Alanine	0.275	0.275
L-Arginine	0.097	0.097
L-Aspartate	0.040	0.040
L-Asparagine	0.042	0.042
L-Cysteine	0.081	0.081
Glycine	0.411	0.411
L-Proline	0.136	0.136
L-Serine	0.117	0.117
L-Tyrosine	0.075	0.075

[1] Experimental substrates were replaced one at a time with 4 concentrations of their corresponding [^{13}C]tracers keeping the basal AA and SCFA mixtures constant. Glucose, L-valine, L-leucine, L-glutamine and L-glutamate were added at final concentrations of 0.25, 0.5, 1, or 2.5 times their basal concentrations.
[2] Incubation media with [$^{13}C_6$]glucose contained 20 mol [^{13}C]tracer/100 mol unlabeled tracee.

The TCA cycle tBDMS derivatives were separated by GC (HP 6890; Agilent) on a fused silica capillary column (HP-5; 30-m × 0.25-mm i.d., 1-μm; Hewlett-Packard) with helium as the carrier gas (1.0 mL/min) and a temperature gradient (100°C for 2 min; 30°C to 160°C; 10°C/min to 250°C/min). The mass spectrometer (HP 5973N Mass Selective Detector, Agilent) was operated in the electron impact mode and selected ion monitoring was performed on the $[M-57]^+$ fragments of the tBDMS derivatives from [M] to [M+n], where n is the number of carbon atoms in the analyte. Ions with mass-to-charge were monitored for lactate 261–264, pyruvate 274–277, ketoisovaleric acid (KIV) 302–307, ketoisocaproic acid (KIC) 316–322, succinate 289–293, oxaloacetate 432–436, and α-ketoglutarate 446–451.

Enrichments were expressed as tracer:tracee ratios (TTR; mol ^{13}C-isotopomer/100 mol ^{12}C-isotopomer). The mole fraction of each ^{13}C-isotopomer was calculated as:

$$^{13}\text{C-isotopomer mole fraction} = [M+i]/([M] + \sum_{n}^{1}[M+n]),$$

where [M + i] is the enrichment of the ^{13}C-isotopomer, [M] is the ^{12}C-isotopomer, and $\sum_{n}^{1}$[M+n] is the sum of all ^{13}C-isotopomers from 1 to n carbons. Crude mass isotopomer distributions were corrected for the difference between theoretical and measured values using the correlation matrix approach (28).

Precursor-product relationships [fractional contribution (FC)] were calculated (29,30) using the general equation:

$$FC_{precursor \rightarrow product} = TTR_{product}/TTR_{precursor},$$

where $TTR_{precursor}$ is the mole fraction of the precursor and $TTR_{product}$ is the mole fraction of the product that unequivocally derives from the precursor. For example, metabolism of [M + 6]glucose in the glycolytic pathway can yield [M + 3]pyruvate and subsequently [M + 3]lactate. Thus, the proportion of pyruvate and lactate flux derived from metabolism of glucose can be calculated as:

$$\text{lactate flux from glucose} = [M+3]\text{lactate}/[M+6]\text{glucose}$$

$$\text{pyruvate flux from glucose} = [M+3]\text{pyruvate}/[M+6]\text{glucose}.$$

Similar precursor-product relationships were applied to determine the contribution of glutamine and glutamate (i.e. M+5) to the fluxes of α-ketoglutarate (M+5), succinate (M+4), and oxaloacetate (M+4), the contribution of valine (M+5) to KIV (M+5) and succinate (M+4) fluxes, and the contribution of leucine (M+5) to KIC (M+5) and acetyl-CoA (i.e. [M+2] α-ketoglutarate) fluxes.

Statistical analysis. After verifying the assumptions of normality and homogeneity of variance, results were analyzed by ANOVA using procedures of SAS (version 8.0, SAS Institute). The isotopomer distributions of metabolic intermediates at the middle levels of media concentration, which represent physiologic concentrations of substrates, were analyzed statistically. Where enrichments were significantly different from zero, precursor-product relationships ($FC_{precursor \rightarrow product}$) were computed and a further statistical analysis was conducted to test for treatment, tissue, substrate concentration, and their interactions.

Data were analyzed by ANOVA for a 3-way factorial design using the MIXED procedure of SAS. The following linear mixed model was employed:

$$Y_{ijk} = \mu + T_i + S_j + C_k + TS_{ij} + TC_{ik} + SC_{ik} + TSC_{ijk} + \varepsilon_{ijk},$$

where $Yijk$ is the observed value for the ith dietary treatment jth tissue and kth substrate concentration, μ is the grand mean, T_i is the treatment effect for the ith treatment, S_j is the tissue effect for the jth tissue, C_k is the concentration effect for the kth substrate concentration TS_{ij}, TC_{ik}, SC_{jk}, and TSC_{ijk} are the interaction effects, and ε_{ijk} is the random error associated with Y_{ijk}. When a significant 3-way interaction was detected, we compared all means using the Tukey-Kramer multiple comparison test. If a 2-way interaction was detected, then means were separated within a diet when the tissue × substrate concentration was significant, within tissue when the diet × substrate concentration was significant, or within substrate concentration when diet × tissue was significant. Only the highest-order interactions are presented in the figures. Otherwise, the main effects of diet, tissue, and concentrations are presented. Data are least square means ± pooled SEM and differences were considered significant at $P \leq 0.05$.

Results

Glucose metabolism. There was considerable appearance of [M+3] pyruvate and [M+3] lactate and although lower ^{13}C-mass isotopomers ([M+1] and [M+2]) were detectable in pyruvate, lactate, and other TCA cycle intermediates, the enrichment of these isotopomers was below (<0.2 mole % excess) the confidence limits of the GC-MS measurement. For DMC, within a diet, the contribution of glucose to pyruvate flux increased from 3 to 9% with increasing glucose concentrations, whereas for REC this contribution remained low (1–2%, **Fig. 1***A*). Within a concentration, for REC from bulls fed the HC diet and for DMC from bulls fed both diets (Fig. 1*B*), a larger proportion of lactate flux derived from glucose (32%) compared with REC from bulls fed the HF diet (15%).

Glutamine and glutamate metabolism. Within a cell type and diet, the proportion of α-ketoglutarate flux derived from glutamine catabolism increased ($P < 0.05$) from 0.2 to 2.7% with increasing glutamine concentrations (**Fig. 2**). Furthermore,

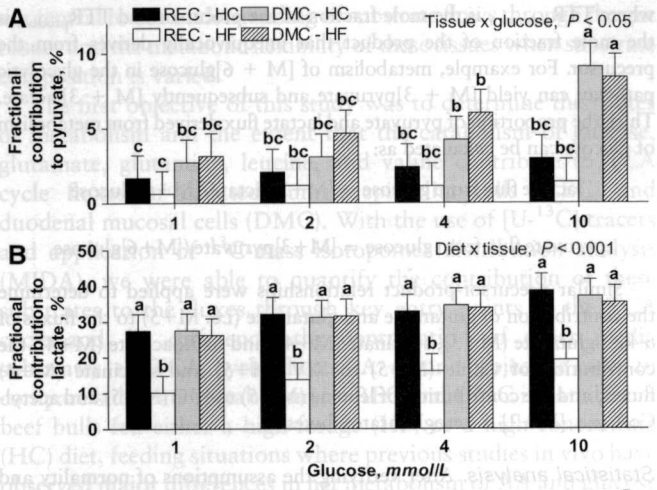

FIGURE 1 The contribution of glucose to the flux of pyruvate (*A*) and lactate (*B*) in REC and DMC cells isolated from bulls fed a HF or HC diet for 4 wk. Isolated cells were incubated with increasing concentrations of [$^{13}C_6$] glucose. Values are means ± pooled SEM, $n = 6$. Means within each diet (*A*) or concentration (*B*) without a common letter differ, $P \leq 0.05$.

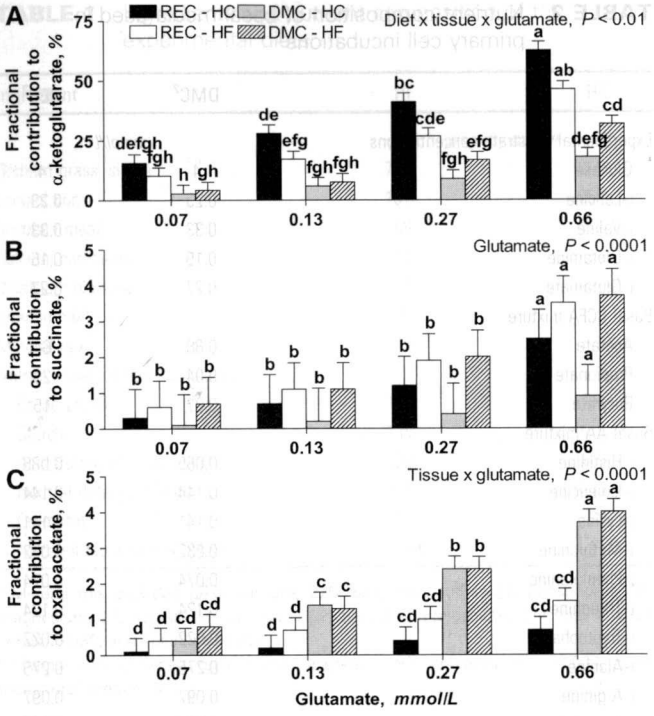

FIGURE 3 The contribution of glutamate to the flux of α-ketoglutarate (*A*), succinate (*B*), and oxaloacetate (*C*) in REC and DMC cells isolated from bulls fed a HF or HC diet for 4 wk. Isolated cells were incubated with increasing concentrations of [$^{13}C_5$] glutamate. Values are means ± pooled SEM, $n = 6$. (*A*) Means without a common letter differ, $P \leq 0.05$. (*B*) Means within a diet and tissue without a common letter differ, $P \leq 0.05$. (*C*) Means within a diet without a common letter differ, $P \leq 0.05$.

there was no detectable ^{13}C-labeling of TCA cycle intermediates and lactate. In contrast to glutamine, there was considerable catabolism of glutamate by both cell types to α-ketoglutarate and other TCA cycle intermediates with increasing glutamate concentrations (**Fig. 3**). However, glutamate accounted for a larger proportion of α-ketoglutarate (Fig. 3*A*) flux in REC (31%) compared with DMC (12%). In addition, for REC, glutamate accounted for a greater proportion of α-ketoglutarate flux (37 vs. 25%) when bulls were fed the HC diet. By contrast, diet did not affect glutamate catabolism to α-ketoglutarate in DMC (12% of flux).

There was appreciable ^{13}C-labeling of other TCA cycle intermediates when media contained [$^{13}C_5$]glutamate and this increased with glutamate concentration. Thus, in addition to α-ketoglutarate, glutamate accounted for a greater ($P < 0.05$) proportion of succinate flux when the glutamate concentration increased, but the proportion of succinate flux derived from glutamate was several-fold less than for α-ketoglutarate (Fig. 3*B*). This latter observation suggests that other substrates in the media continued to be metabolized via succinyl-CoA. Likewise, although glutamate accounted for an increasing proportion of oxaloacetate flux for DMC, the contribution from glutamate

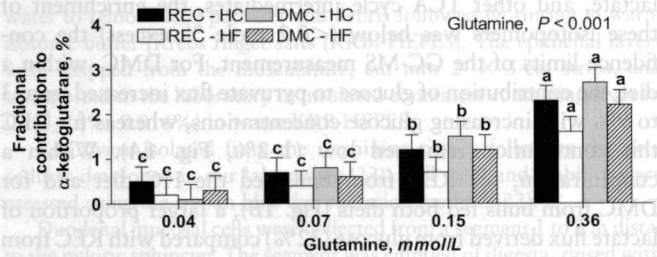

FIGURE 2 The contribution of glutamine to the flux of α-ketoglutarate in REC and DMC cells isolated from bulls fed a HF or HC diet for 4 wk. Isolated cells were incubated with increasing concentrations of [$^{13}C_5$] glutamine. Values are means ± pooled SEM, $n = 6$. Means within a diet and tissue without a common letter differ, $P \leq 0.05$.

catabolism was also less than its contribution to α-ketoglutarate flux. In contrast to DMC, the contribution of glutamate to oxaloacetate flux was less for REC ($P < 0.05$) and increasing media glutamate did not alter this contribution (Fig. 3*C*). Of particular note, there was no or very little dilution of ^{13}C between succinate and oxaloacetate for DMC and REC, suggesting only minor contributions of other substrates (e.g. aspartate, asparagine, flux from pyruvate) metabolized for entry between these points of the TCA cycle.

Valine and leucine metabolism. As far as we know, the only substrates that are metabolized to KIV and KIC are the AA valine and leucine, respectively. The flux of these keto-acids that did not derive from media valine or leucine must arise from cell protein turnover. The mean contribution of valine to KIV flux in REC and DMC incubations (**Fig. 4**) increased ($P < 0.05$) from 26 to 73% at the highest valine concentration. In addition, irrespective of diet, the contribution of valine to KIV flux was greater for REC (58%) than for DMC (40%). For leucine, its contribution to KIC flux was 44% for REC from bulls fed the HC diet compared with 35% for DMC. For REC and DMC from bulls fed the HF diet, 36% of KIC flux derived from media leucine (**Fig. 5**). Of particular note, ^{13}C from valine and leucine was not detectable in TCA intermediates and lactate.

Discussion

Two design aspects of the current study are worth noting. First, in the current study, cell media contained [U-^{13}C]-labeled substrates

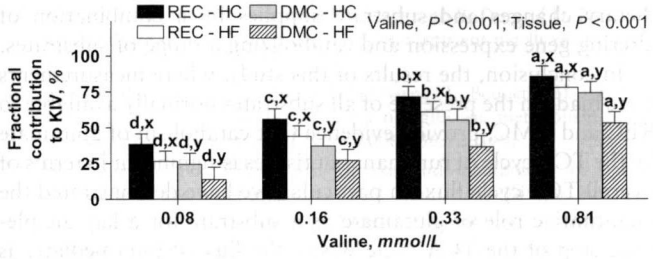

FIGURE 4 The contribution of valine to the flux of ketoisovalerate in REC and DMC cells isolated from bulls fed a HF or HC diet for 4 wk. Isolated cells were incubated with increasing concentrations of [$^{13}C_5$] valine. Values are means ± pooled SEM, $n = 6$. Means without a common letter differ, $P \leq 0.05$ (a, b, main effect of valine concentration; $P \leq 0.05$; x, y, main effect of tissue; $P \leq 0.05$).

and the ^{13}C-MIDA in end products and TCA cycle intermediates determined by GC-MS analysis. This approach provided rich information on the routes of metabolism of the substrates and, in particular, their unequivocal contributions to the flux at key points in the TCA cycle. Thus, rather than quantifying the catabolic fates of the substrates, we quantified their contribution to energy yielding and synthetic pathways of cell metabolism. The second design aspect is that we purposely formulated the incubation media to reflect the composition and concentration range of all substrates that REC and DMC would likely be exposed to in vivo (31–33). This contrasts with previous studies in vitro where the metabolic fates of AA, glucose, and SCFA (acetate, propionate, and butyrate) have been assessed either when they are the sole substrate in the media at supraphysiological concentrations (e.g. >2 mmol/L) or in combination with another single substrate (16–18,23,34–37). Catabolism under these conditions certainly will differ from normal where all substrates are present at concentrations that reflect those found in blood or within the gut lumen.

In the current study, the use of macronutrients in intermediary metabolic pathways was detailed in REC and DMC cultures by use of specific ^{13}C-labeled substrates normally available to the absorptive aspect of the cells lining the rumen and small intestines in vivo, and muscularis cells were removed. In addition, the cell isolation and incubation conditions employed in our laboratory resulted in minor, if any, contributions of anaerobic bacteria to the overall metabolism in vitro. In this respect, the results should closely reflect metabolism of the substrates on first pass through the gut tissues. We set out to test whether REC and DMC possessed metabolic plasticity to catabolize AA. We hypothesized that this flexibility was dependent upon the initial point(s) of entry of AA to the TCA cycle and diet conditions. Other metabolic substrates (glucose and SCFA) would presumably compete with AA for the common entry points to the TCA cycle and, therefore, spare AA from catabolism.

The first observation we highlight is that the extent of glutamate and glutamine catabolism via the TCA cycle of REC and DMC differed. Oxidation to CO_2 has been shown to be the primary catabolic fate of both of these AA in vitro (cattle and sheep) (34–36) and in vivo (pigs) (6,8). Because both AA share the same catabolic pathway, we expected that glutamine would contribute substantially to α-ketoglutarate flux in REC and DMC based on our observations with isolated gut cells from sheep that CO_2 production from glutamine exceeds that from glutamate at similar concentrations in vitro (36). We hypothesized that metabolic plasticity of the GIT is dependent upon the initial point(s) of entry of AA to the TCA cycle. Our data indicated that this is unlikely. Glutamate was the largest contributor to TCA cycle intermediate fluxes and its carbon skeleton accounted for 4–63% of α-ketoglutarate flux. The contribution of glutamate to α-ketoglutarate flux increased with glutamate supply and it was higher in REC (13–54%) compared with DMC (4–25%). By contrast, the contribution of glutamine to α-ketoglutarate flux did not exceed 3% for REC and DMC.

Quantitatively, catabolism of glutamate for energy by pig intestinal tissues is greater than for glutamine (38). The authors of that study suggested that glutamine catabolism through deamidation may reflect a requirement for glutamate and not for glutamine per se. An observation that further supports this view is that in isolated sheep enterocytes, addition of glucose to the media did not reduce glutamate oxidation to CO_2. This suggested to us that glutamate catabolism might be obligatory for these cells (36). Conversely, glucose addition to rumen papillae and DMC incubation media reduced CO_2 production from glutamine (34–36). With respect to the contribution of glutamate to TCA cycle intermediate fluxes, it is important to note that catabolism of glutamate accounted for less of the succinate flux (1–4%) than its contribution to α-ketoglutarate flux (12–37%). In addition, even with increasing media glutamate, this differential in contribution was nearly maintained, not reduced. Thus, not only did other media substrates (e.g. propionate, threonine, isoleucine, and methionine) make larger contributions to succinate flux than glutamate, but their metabolism through succinyl-CoA was also largely maintained even with increasing glutamate supply.

A second observation was that the catabolism of glucose to pyruvate and lactate did not lead to appreciable entry of glucose carbon into the TCA cycle. Our data indicate that a greater proportion of pyruvate and lactate fluxes in REC from bulls fed the HC diet derived from glucose compared with REC from bulls fed the HF diet. Thus, glucose may spare catabolism of other substrates that are metabolized via pyruvate, in particular when higher starch diets are fed. Previous reports have also shown that glucose is oxidized to CO_2 by isolated enterocytes (34,36) and rumen papillae (35). In the latter study, glucose uptake and oxidation to CO_2 increased for rumen papillae isolated from steers fed a 90%-concentrate diet compared with those from steers fed alfalfa hay.

There is evidence to suggest that the high rate of glycolysis in fast growing cells such as GIT, immune, and cancer cells occurs to support ribose production through the pentose phosphate pathway (39). Therefore, lactate production may represent a salvage mechanism for glucose that is catabolized in the glycolytic pathway. Our data also suggest that CO_2 production

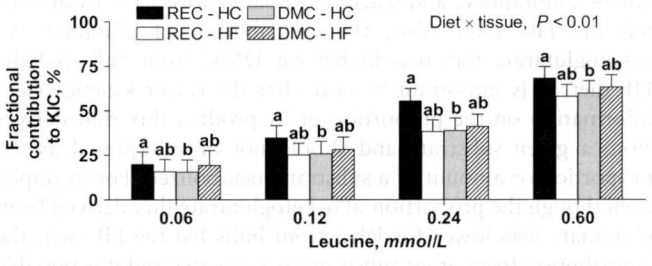

FIGURE 5 The contribution of leucine to the flux of ketoisocaproate in REC and DMC cells isolated from bulls fed HF or HC diet for 4 wk. Isolated cells were incubated with increasing concentrations of [$^{13}C_6$] leucine. Values are means ± pooled SEM, $n = 6$. Means within each concentration without a common letter differ, $P \leq 0.05$.

from glucose observed by others (34,35,40) could have occurred in the oxidative arm of the pentose cycle. There are no studies in ruminants that have compared GIT oxidation of glucose via the pentose cycle and the TCA cycle. However, in pig enterocytes, glucose oxidation via the pentose cycle accounted for ~90% of the CO_2 production from glucose (37). It is possible that a similar mechanism occurs in the GIT of ruminants given the limited ^{13}C-labeling of TCA intermediates observed in the current study using [$^{13}C_6$]glucose.

The third observation we highlight relates to the catabolism of the BCAA. Although we observed a significant and concentration-dependent increase in the contribution of leucine (17–63%) and valine (19–82%) to KIC and KIV fluxes, we did not detect ^{13}C-labeling of TCA cycle intermediates that would indicate further catabolism (oxidation) of these AA. In studies in sheep and cattle where ^{13}C or ^{14}C-leucine was infused i.v., there was a net release of ^{13}C- or ^{14}C-KIC by the PDV tissues (13,41–43). Moreover, these studies observed that 8–22% of arterial leucine extracted by the PDV was catabolized to CO_2 (13,42,43), whereas <5% of leucine was oxidized when the tracer was infused via the intestinal lumen route (42–44). Therefore, these data seem to suggest that PDV oxidation of leucine, and perhaps other BCAA, occurs primarily by the cells of PDV tissues that are exposed to arterial blood, whereas little oxidation of BCAA occurs by the luminal-facing mucosal cells. Our results with isolated REC and DMC (i.e. luminal-facing cells) are compatible with observations in vivo that metabolism of leucine and valine to CO_2 is not a significant catabolic fate of these AA when they are taken up from the lumen of the GIT. Similar results have also been reported for isolated pig jejunal mucosal cells where 66–71% of the BCAA taken up by these cells was released as the corresponding keto-acid (45).

Previous studies have shown that net metabolism of AA, glucose, and SCFA by the PDV of growing sheep and beef steers is greater when animals are fed a HF compared with a HC diet at equal dry matter intakes (19,20). Based on transcript analysis of the isolated cells from the bulls in the current study, mRNA abundances of branched-chain ketoacid dehydrogenase (EC 1.2.4.4), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and glutamate-oxaloacetate transaminase-2 (EC 2.6.1.1) were greater for REC from bulls fed the HF diet, whereas mRNA abundances of glutaminase (EC 3.5.1.2), glutamate dehydrogenase-1 (EC 1.4.1.3), and sodium glucose cotransporter-1 were not altered by diet for REC and DMC (21). In parallel, the tracer substrates chosen in the present study were selected to evaluate the metabolic flux of these substrates through these enzyme pathways. In comparison to the gene expression results, we observed that the REC from bulls fed the HF diet derived a lower proportion of lactate, KIC, and TCA fluxes from catabolism of glucose, glutamate, and leucine compared with REC from bulls fed the HC diet. Also, the contribution of glutamate to α-ketoglutarate flux was higher for DMC from bulls fed the HF diet. It is important to note that the tracer kinetics yield information on the proportion of by-product flux that derives from a given substrate and should not be interpreted as the proportion or amount of a substrate metabolized. For example, even though the proportion of α-ketoglutarate flux derived from glutamate was lower for REC from bulls fed the HF diet, the contribution from other substrates was greater and it is possible that the overall flux and, therefore, metabolism by the REC increased with the HF diet. Indeed, oxygen consumption by the whole GIT is greater in beef steers fed a HF compared with a HC diet (4). The present data also provide evidence that tissues of the GIT of ruminants are capable of metabolically adapting to dietary changes and substrate supplies by a combination of altering gene expression and catabolizing a range of substrates.

In conclusion, the results of this study, where measurements were made in the presence of all substrates normally available to REC and DMC, provide evidence that catabolism of glutamate by the TCA cycle of ruminant gut tissues is significant in terms of overall TCA cycle flux. In particular, we have demonstrated the quantitative role of glutamate as a substrate for a key anaplerotic step of the TCA cycle where the flux of intermediates is critical to ensure continued fluxes through cataplerotic pathways. By contrast, the contribution of glutamine to the TCA cycle via α-ketoglutarate was much lower than for glutamate. Our data also suggest that despite the large contribution of glucose to pyruvate and lactate fluxes, there was limited entry of glucose carbon into the TCA cycle. The glycolytic degradation of glucose may play a role in providing precursor substrate to other metabolic pathways, in particular the pentose cycle. We believe that, in ruminants, the catabolism of glucose primarily to lactate serves a role in preserving glucose carbon skeletons from further catabolism by the GIT, in particular as 3-carbon units that are subsequently recycled by the liver for resynthesis of glucose.

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